Tributyrin and lactitol synergistically enhanced the trophic status of the intestinal mucosa and reduced histamine levels in the gut of nursery pigs

A. Piva, A. Prandini, L. Fiorentini, M. Morlacchini, F. Galvano and J. B. Luchansky

J Anim Sci 2002. 80:670-680.

Tributyrin and lactitol synergistically enhanced the trophic status of the intestinal mucosa and reduced histamine levels in the gut of nursery pigs[1,2,3,4]

A. Piva*[5], A. Prandini†, L. Fiorentini†, M. Morlacchini‡, F. Galvano§, and J. B. Luchansky¶

*DIMORFIPA, University of Bologna, 40064 Ozzano Emilia, Bologna, Italy; †ISAN, Facoltà di Agraria, Universitá Cattolica del S. Cuore, 29100 Piacenza, Italy; ‡Centro Ricerche per la Zootecnia e l'Ambiente, S. Bonico, 29100 Piacenza, Italy; §Dipartimento STAFA, Università di Reggio Calabria, 89121 Reggio Calabria, Italy; and U.S. Department of Agriculture, Agricultural Research Service, 600 East Mermaid Lane, Wyndmoor, PA 19038.

ABSTRACT: This study determined whether tributyrin and lactitol could synergistically facilitate the transition from milk to solid feed in nursery pigs. At 21 d after birth, 64 piglets were moved from the piggery to a production barn and fed a medicated diet. At 28 d after birth, the piglets were weighed and allotted into four groups and fed a standard nonmedicated diet (control) or the control diet with tributyrin (butanoic acid 1,2,3-propanetriyl ester; 10 g/kg), or with lactitol (β-D-galactopyranosyl-(1→4)-D-sorbitol; 3 g/kg), or with tributyrin (10 g/kg) plus lactitol (3 g/kg). On d 0, 14, and 42 after being fed the control or experimental diets, the animals were weighed, and animal health, feed intake, and feed conversion ratio were determined. On d 42, four piglets from each treatment were killed to measure the empty and full weight of the gut, as well as the weights of the liver and kidneys. The jejunum and cecum were sampled to analyze the luminal concentrations of lactic acid; short-chain fatty acids; and mono-, di-, and polyamines and to assess the mucosal status. Mortality after 42 d ranged from 19% for animals fed the control diet, to 6% for animals fed the tributyrin or lactitol diets, and to 0% for animals fed the tributyrin+lactitol diet. After 14 d, the ADG was 127% greater ($P < 0.05$) in animals fed the tributyrin+lactitol diet than in animals fed the control or tributyrin diets. After 42 d, animals fed the tributyrin+lactitol diet were heavier ($P < 0.05$) than animals fed the tributyrin diet. At slaughter, no differences ($P > 0.05$) in organ weights were observed. With the exception of animals fed the lactitol diet, wherein cecal lactic acid levels increased threefold ($P < 0.01$), the luminal concentrations of lactic acid and short-chain fatty acids were not different ($P > 0.05$). Among the various amines analyzed, the only response ($P < 0.05$) was a 66 and 49% decrease in histamine levels in the jejunum and cecum, respectively, in animals fed the tributyrin+lactitol diet compared to the control diet. In the jejunum of animals fed the lactitol or tributyrin+lactitol diets, the length of the villi was increased by 12% ($P < 0.05$) compared to animals fed the control diet, whereas the tributyrin diet did not have any effect on the villi ($P > 0.05$). In the cecum, the depths of the crypts were reduced ($P < 0.001$) by 18% in animals fed the lactitol diet and 45% in animals fed the tributyrin or tributyrin+lactitol diets compared to animals fed the control diet. In conclusion, a diet containing tributyrin and lactitol as nutribiotics resulted in lower histamine levels in the jejunum and cecum, as well as longer jejunal villi and shallower cecal crypts.

Key Words: Butyric Acid, Intestinal Mucosa, Lactitol, Pigs

 J. Anim. Sci. 2002. 80:670–680

Introduction

Research on promoting intestinal functionality through manipulation of nutrient availability and microbial activity is fueled by the mandate to find alter-

[1]Patent No: U.S. 6,217,915 (Luchansky and Piva, 2001).

[2]Presented at the Joint Annual Meeting of the American Society of Animal Science and of the American Dairy Science Association in Baltimore, MD, on July 26, 2000.

[3]Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[4]We gratefully acknowledge Gianfranco Piva (director, Institute of Food Science and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy) for his coordination of the feeding trial at the CERZOO and Bruno Volpi (Università Cattolica del Sacro Cuore, Piacenza, Italy) for his technical assistance with the SEM analyses.

[5]Correspondence: Via Tolara di Sopra, 50 (phone: +39-051-79-2883; fax: +39-051-79-2869; E-mail: apiva@alma.unibo.it).

Received December 4, 2000.
Accepted October 2, 2001.

natives to antibiotic growth promoters. Alternatives include diet processing with the use of feed supplements such as probiotic cultures, organic acids, and(or) prebiotic indigestible oligosaccharides (Nousiainen and Setälä, 1992; Partanen and Mroz, 1999; Piva and Morelli, 1997).

Most of these strategies function primarily by affecting the intestinal flora; however, it may also be beneficial to provide specific nutrients to directly feed gut tissues (Luchansky, 2000). In this respect, it may be favorable to increase the availability of short-chain fatty acids (**SCFA**) through the modulation of the anaerobic fermentation in the gut or through direct dietary supplementation with SCFA and(or) with precursors of SCFA. In particular, *n*-butyrate has elicited considerable interest because it is the main energy substrate for colonocytes; approximately 75% of the oxygen consumed by colonocytes is due to *n*-butyrate metabolism (Roediger, 1980). Moreover, *n*-butyrate is a potent antiproliferative and differentiation agent in various cancer cell lines (Schroder et al., 1999). As an indirect approach to increase the production of SCFA in the hindgut and, as such, of *n*-butyrate, the diet can be supplemented with indigestible oligosaccharides (Levrat et al., 1991; Tsukamura et al., 1998). As an example, the fermentation of lactitol in the cecum increased the concentration of *n*-butyrate from 142 to 230% when added to low- or high-fiber diets, respectively (Piva et al., 1996).

The purpose of the present work was to determine whether a combination of tributyrin, a generally recognized as safe (GRAS) flavoring substance (CFR, 1997), as a dietary source of butyric acid, and lactitol, a noncariogenic dietary sugar alcohol (CFR, 2000), as an in situ source of SCFA, could enhance the health status and growth performance of swine.

Materials and Methods

Animals, Diets, and Facilities

The experiment monitored the effect of diet on 64 weaned piglets ([Large White × Landrace] × Duroc; Centro Ricerche per la Zootecnia e l'Ambiente (CERZOO) S. Bonico, Piacenza, Italy). At 21 d after birth, the piglets were transported from the piggery to the production barn. Before the trial was initiated, the piglets were kept in flat-deck cages for 7 d to monitor their health status. During this 7-d adaptation period, the animals were fed a commercially available, medicated diet (containing [mg/kg diet]: chlortetracycline, 1,000; and spiramycin, 400; Mangimi Ferrari, Piacenza, Italy) as a prophylactic to minimize the negative consequences of stress due to transfer from the piggery to the production barn. The piglets always had free access to water and feed.

At 28 d after birth, the piglets (5.9 ± 0.15 kg live weight) were allotted randomly into the following four experimental dietary groups (16 pigs/diet with 4 pigs/pen) according to their initial weight, gender (females and castrated males), and litter and were provided with pelleted experimental feeds (Table 1): 1) control diet; 2) control diet supplemented with tributyrin (10 g/kg; butanoic acid 1,2,3-propanetriyl ester; Chemical Abstract number 60-01-5; Fluka Chemie AG, CH-9471 Buchs, Switzerland); 3) control diet supplemented with lactitol (3 g/kg; (β-D-galactopyranosyl-(1→4)-D-sorbitol; Chemical Abstract number 81025-04-9; Purac biochem bv, 4200 AA Gorinchem, Holland); and 4) control diet supplemented with a patented nutribiotic (Luchansky and Piva, 2001) containing tributyrin plus lactitol (10+3 g/kg). Room temperature was set at 26 to 27°C and recorded continuously in each room. Lighting was natural (November to December).

On d 0, 14, and 42 after the feeding trial was begun, the animals were individually weighed, and feed consumption and feed conversion ratio were determined for each pen. Animal health was monitored throughout the duration of the study by a veterinarian in charge of animal health and welfare at the CERZOO. After 42 d, two castrated males and two females from each dietary treatment were killed to measure the empty and full weights of the stomach, cecum, and colon, as well as the weights of the liver and kidneys. Animals were killed under the supervision of the veterinarian at the CERZOO, by stunning with a captive bolt followed by complete bleeding. Within 20 min after death, the lumen and the mucosa from the middle section of the jejunum and from the cecum were sampled (according to Piva and Morelli, 1997) to determine the levels of mono-, di-, and polyamines and SCFA, and to observe the morphology of these tissues using scanning electron microscopy.

The present study was conducted in accordance with the published guidelines for good laboratory practices (Directives No. 88/320/EEC and No. 90/18/EEC), and animal welfare and protection (Directive No. 86/609/EEC and Italian Law Act Decreto Legislativo No. 116, issued on January 27, 1992). The research farm CERZOO, where the study was conducted, is good laboratory practices-certified and is authorized to perform animal studies according to Section 12 of the above-indicated Act No. 116 by the Italian Ministry of Health (Decreto Ministeriale No. 253/95-A, issued on August 18, 1995). In addition, the ethical committee of the ISAN reviewed and approved the experimental protocol.

Chemical Analyses of Feed and Intestinal Contents

Analyses of the DM, crude protein, ether extract, crude fiber, ash, and starch contents of the feed were performed according to methods approved by the Association of Official Analytical Chemists (AOAC, 1990). The SCFA and lactic acid concentrations from the jejunum and cecum were analyzed by gas chromatography (Varian 3400; Varian Analytical Instruments, Sun-

Table 1. Composition of diets (as-fed basis)

Ingredient	Diet[a]				Chemical composition	Diet[a]			
	CTR	TRB	LCT	TRB+LCT		LCT	TRB	LCT	TRB+LCT
	g/kg diet					g/kg diet			
Corn			373.3		Dry matter	892.0	890.8	893.7	890.6
Wheat			130.0			g/kg DM			
Barley			200.0		Crude protein	199.3	204.2	201.6	200.4
Soybean meal			165.0		Ether extract	53.6	63.4	54.7	62.0
Meat meal			20.0		Crude fiber	38.8	38.5	42.1	39.1
Fish meal			30.0		Ash	62.3	60.8	62.9	60.6
Tallow			20.0		Ca	10.7	10.7	10.7	10.7
Dried whey			30.0		P	7.5	7.5	7.5	7.5
L-Lysine HCl			2.9		Starch	487.4	469.8	474.3	474.1
DL-Methionine			0.8			MJ/kg DM			
L-Tryptophan			0.5		Digestible energy[c]	16.09	16.11	16.08	16.10
L-Threonine			0.3		Net energy[d]	12.00	12.02	12.00	12.02
$CaCO_3$			7.0			g/kg DM			
$CaHPO_4$			14.0		Lysine	13.5	Leucine		17.1
NaCl			2.2		Methionine	4.6	Isoleucine		9.1
Premix[b]			4.0		Meth.+Cystine	8.1	Valine		10.3
Tributyrin	—	10.0	—	10.0	Threonine	8.1	Histidine		5.2
Lactitol	—	—	3.0	3.0	Tryptophan	2.7	Arginine		12.4
					Phenylalanine + Tyrosine	16.7			

[a]Abbreviations: CTR, control; TRB, tributyrin; LCT, lactitol.
[b]Supplying (mg/kg of diet): retinyl palmitate, 4.8; cholecalciferol, 800; all-*rac*-α-tocopherol acetate, 24; menadione, 2.72; thiamine, 2.2; riboflavin, 4.4; pyridoxine, 2.72; cyanocobalamin, 0.022; niacin, 26; biotin, 0.12; pantothenic acid, 11; choline, 548; Co, 0.44; Fe, 64; Cu, 33; Zn, 108; Mn, 28; I, 1.64; Se, 0.1; DL-methionine, 108; L-Lysine, 120.
[d]According to Noblet et al. (1994).
[e]According to Morgan and Whittemore (1982).

nyvale, CA) using a Carbopack B-DA/4% CW 2M, 80/120 packed column (Supelco; Sigma Aldrich s.r.l., Milano, Italy) according to Fussell and McCailey (1987). Prior to injection, the intestinal contents were centrifuged (6,000 × *g*, 15 min, 4°C), and 2 mL of the supernate was mixed with 1 mL of pivalic acid (98% pure), 1 mL of ossalic acid (99.8% pure), and 250 μL of formic acid (99% pure) (Fussel and McCailey, 1987).

The hydrochloride salts of mono-, di-, and polyamines were purchased from Sigma Chemical Co. (St. Louis, MO). Spectrophotometric-grade acetonitrile was purchased from Merck KgaA (Darmstadt, D64271, Germany). Mono-, di-, and polyamines were separated and quantified by HPLC with fluorimetric detection as follows: frozen intestinal lumen contents were thawed on ice, homogenized with 10 mL of 0.2 *M* perchloric acid in the presence of 16 n*M* 1,7-diaminoheptane (final concentration) as an internal standard, maintained for 1 h at 4°C, rehomogenized under the previously described conditions, and then centrifuged at 9,000 × *g* for 20 min at 4°C. Amines were derivatized according to the method of Flores and Galston (1982). Portions of the supernate were buffered by a double volume of a saturated solution of sodium carbonate and were added to 4 vol of dansyl chloride (7.5 mg/mL) in acetone. The mixture was incubated in a thermal reaction block at 60°C for 1 h in the dark. A 100-μL volume of proline (100 mg/mL in water) was added to the mixture to remove the excess dansyl chloride. After 0.5 h, the amines were extracted with 500 μL of toluene with vigorous vortexing for 30 s to separate the mixture into aqueous and organic phases. The extraction procedure was performed twice. The upper organic phase, containing the amines, was removed and completely dried under nitrogen. The polyamine residue was dissolved in 1 mL of a 1:1 water:acetonitrile solution, ultrafiltered through nylon membranes (0.45-μm pore syringe filter; Step-Bio, Cameo 13N, Bologna, Italy) and assayed immediately or stored for ≤ 1 wk at −20°C. The HPLC was performed using two solvent metering pumps (PU-980, JASCO Corp., Tokyo, Japan) and an autosampler (AS-950, JASCO). Samples were injected into a fixed 100-μL loop for loading onto a reverse phase C18 column (LiChroCART 125-4 Superspher 100 RP-18, Merck). Samples were eluted from the column with a programmed water:acetonitrile (vol/vol) solvent gradient, changing from 40 to 70% in 17 min at a flow rate of 1 mL/min. Elution was completed within 26 min. The column was washed with 100% acetonitrile for 14 min, and reequilibrated with 40% acetonitrile for 10 min before the next sample was injected. Eluates from the column were detected by an attached fluorescence spectrophotometer (FP-920, JASCO). For dansyl amines, an excitation wavelength of 340 nm was used with an emission wavelength of 545 nm (Seiler and Knodgen, 1978). The lim-

its of detection for the various amines were putrescine, 0.023; cadaverine, 0.022; histamine, 0.48; tyramine, 0.068; spermidine, 0.012; and spermine, 0.007 p*M*.

Scanning Electron Microscopy of Intestinal Mucosa

Intestinal mucosa from the middle section of the jejunum and from the cecum of all sacrificed animals were examined by scanning electron microscopy. Tissue samples (10 mm^2 in size) were rinsed with a PBS solution (0.15 *M*; made of [g/L] $Na_2HPO_4 \cdot 12H_2O$, 2.89; KH_2PO_4, 0.2; KCl, 0.2; NaCl, 8), fixed in glutaraldehide (2.5% v/v) in cacodilate buffer (0.15 *M*) for 3 h at 4°C, rinsed with cacodilate buffer (0.15 *M*), and postfixed in osmium tetraoxide at 1% (wt/vol) for 1 h at 4°C. Samples underwent progressive dehydration using 75, 85, 95, and 100% ethanol for 12 h each at room temperature and subjected to drying under vacuum. Tissues were examined at 25 kV with a scanning electron microscope (HITACHI S-2300; Nanovision, Milano, Italy) to measure enterocyte and villus length, cecal crypt depth, and jejunal and cecal mucosal thickness. These measures were taken according to Pluske et al. (1996c). Images were captured using a Kevex 4416-4561 processor (Kevex Instruments; San Carlos, CA) and were processed with the Digital Image Processing System (Point electronic GmbH, Halle, Germany).

Statistical Analyses

Growth and feed efficiency data were subjected to analysis of variance (ANOVA) using the GLM procedures of SAS (SAS Inst. Inc., Cary, NC). The differences among group means were compared using Student's *t*-test based on the variance derived from the ANOVA. The SCFA, amines, and histology data were subjected to one-way ANOVA with Bartlett's test for equal variances and with the Newman-Keuls posttest, using the program GraphPad Prism (GraphPad Software, San Diego, CA) under Windows 95 (Microsoft, Redmond, WA). Differences were considered as significant at $P < 0.05$.

Results

Animals Health Status

No untoward clinical conditions were observed during the 7-d adaptation period by the veterinarian in charge of animal welfare at the CERZOO. As such, no medical interventions or treatments were performed. This short adaptation period using a medicated diet was implemented to allow us to measure the efficacy of the dietary treatments for stressed animals. Typically, the withdrawal of a medicated diet is often associated with increased weight loss and mortality. During the 42 d of the feeding trial, most animals in each treatment group intermittently experienced some form of mild diarrhea, presumably as a consequence of the relatively short, 7-d adaptation period, during which the animals adjusted to the production barn and to solid feeding. Necropsy of the five piglets that died during the feeding trial indicated the occurrence of enteritis, with particular inflammation in the small intestine. The veterinarian attributed death to the stress that the animals were exposed to during weaning and transport, as well as to the stress experienced during grouping and weighing. The comparative morbidity and mortality among animals in the dietary treatment groups are listed in Table 2. Animals fed the control diet experienced the greatest mortality, followed by animals fed the tributyrin or lactitol diets. No animals fed the diet containing both tributyrin and lactitol died.

Animal Performance

Feed consumption was not affected by any of the dietary treatments in either the d 0 to 14 ($P = 0.71$) or 0 to 42 ($P = 0.40$) observation period (Table 3). During the first 14 d of the feeding trial, the tributyrin diet did not affect the growth response ($P > 0.05$) compared to the control diet. Animals receiving the lactitol diet displayed only a tendency toward higher live weight (+13%, $P = 0.12$) and higher daily weight gain (+65%, $P = 0.15$) than control animals. Statistically significant differences were achieved only when tributyrin and lactitol were fed in combination; animals fed the tributyrin+lactitol diets displayed a higher live weight (+19%, $P = 0.048$) and a higher daily weight gain (+127%, $P = 0.007$) than control animals. The average feed efficiencies were 360, 316, 507, and 621 (grams of gain/kg feed, $P = 0.09$) for the control, tributyrin, lactitol, and tributyrin+lactitol diets, respectively; however, the differences among diets were not significant due to the wide variation in the weight of the animals fed the control or tributyrin diets. Such differences were reflected in the large SE observed for the feed conversion ratio data.

Between d 14 to 42 after the feeding trial began, animals fed the tributyrin diet displayed a tendency of lower final live weight (–20%, $P = 0.054$) and average daily gain (–34%, $P = 0.08$) than animals on the control diet. Feeding animals the lactitol or tributyrin+lactitol diets did not modify these growth parameters ($P > 0.05$). The feed efficiency throughout the 42 d of the trial was lower only for animals receiving the tributyrin diet (–20%, $P < 0.05$).

At slaughter, organ weights were within the expected normal ranges, and no alterations were observed among the internal organs of the castrated male or female animals killed for each treatment group. Liver, right and left kidney, and the full and empty stomach, cecum, and colon averaged 64.37, 4.94, 4.99, 127.75, 27.27, 14.96, 5.44, 67.94, and 34.58 g/kg of metabolic live weight, respectively, with no differences ($P > 0.05$) among dietary treatments (data not shown).

Table 2. Incidence of mortality and weight loss of piglets

Item	Diet[a]			
	CTR	TRB	LCT	TRB+LCT
No. of pigs at the beginning of the study[b]	16	16	16	16
Day 0–14 on test				
Mortality	1	1	0	0
No. pigs that lost weight[c]	5	4	2	1
Day 0–42 on test				
Mortality	3	1	1	0
No. pigs that lost weight	1	3	0	0
Mortality incidence, %	18.75	6.25	6.25	0

[a]Abbreviations: control diet (CTR) with or without tributyrin (TRB, 10 g/kg) and(or) lactitol (LCT, 3 g/kg).
[b]28 d of age.
[c]Calculated as the difference between the final and initial weights for either d 0 through 14 of the trial or d 0 through 42 of the trial and expressed as the number of animals that weighed less at d 14 or 42 than at d 0.

The average live weight of killed pigs was 16.5 ± 2.7 kg, with no differences among treatments ($P = 0.80$).

Short-Chain Fatty Acids and Amines in the Jejunum and Cecum

The DM of the intestinal contents in the jejunum (102 ± 40 g/kg; $P = 0.50$) or cecum (105 ± 24 g/kg; $P = 0.39$) were not affected by supplementation of the diets with tributyrin and lactitol, alone or in combination. The jejunal contents from the two castrated males and two females from each dietary treatment showed no differences ($P > 0.05$) in the concentrations of acetic, propionic, iso- or *n*-butyric and valeric acids (Table 4). However, animals fed the lactitol diet had increased ($P < 0.05$) lactic acid concentrations (611.2 μmol/g DM) compared to animals fed the tributyrin (201.7 μmol/g DM) or tributyrin+lactitol (243.7 μmol/g DM) diets, but were not different from animals fed the control diet (441.9 μmol/g DM; $P = 0.24$). Concentrations of total SCFA were not different ($P > 0.05$) among dietary treatments. Lactic acid concentrations in the cecum were threefold higher ($P < 0.01$) in animals fed the lactitol diet than in animals fed the other dietary treatments.

Levels of mono-, di-, and polyamines in the jejunum and cecum did not differ ($P > 0.05$) among animals due to the dietary treatment (Table 5). However, concentrations in the cecum were higher than in the jejunum for cadaverine (4.2 ± 2.8 μmol/g DM vs 1.6 ± 1.3 μmol/g DM; n = 16; $P < 0.01$), putrescine (4.1 ± 2.4 μmol/g DM vs 0.8 ± 0.5 μmol/g DM; n = 16; $P < 0.001$), and spermidine (1.6 ± 0.5 μmol/g DM vs 0.4 ± 0.2 μmol/g DM; n = 16; $P < 0.001$). Concentrations of tyramine and spermine did not differ ($P > 0.05$) between the jejunum and cecum (1.4 ± 1.2 μmol/g DM and 1.1 ±

Table 3. Piglet growth performance

Item	Diet[a]			
	CTR	TRB	LCT	TRB+LCT
No. of pigs d 0[b]	16	16	16	16
Initial live weight on d 0, kg	5.9 ± 0.3	5.9 ± 0.3	6.0 ± 0.3	5.9 ± 0.4
Day 14 on test				
No. of pigs	15	15	16	16
Live weight, kg	7.0 ± 0.4[c]	7.0 ± 0.4[c]	7.9 ± 0.4[cd]	8.3 ± 0.4[d]
Avg daily gain, g/d	81 ± 29.2[c]	77 ± 26.1[c]	134 ± 21.7[cd]	184 ± 21.5[d]
Avg daily intake, g/d[e]	250 ± 18.3	240 ± 17.0	265 ± 3.7	280 ± 6.5
Gain/feed, g/kg	360.2 ± 130.1	316.3 ± 77.2	507.1 ± 68.4	621.0 ± 22.7
Day 42 on test				
No. of pigs	13	15	15	16
Live weight, kg	13.6 ± 0.9[cd]	10.9 ± 1.0[c]	14.3 ± 0.9[d]	14.8 ± 0.9[d]
Avg daily gain, g/d	180 ± 22.2[cd]	119 ± 22.5[c]	201 ± 19.6[d]	213 ± 16.5[d]
Avg daily intake, g/d	365 ± 21.4	299 ± 19.1	370 ± 16.8	362 ± 8.2
Gain/feed, g/kg	527.0 ± 44.1[c]	406.4 ± 39.0[d]	543.5 ± 35.3[c]	565.7 ± 8.2[c]

[a]Control diet (CTR) without or with tributyrin (TRB, 10 g/kg) and(or) lactitol (LCT, 3 g/kg).
[b]28 d of age.
[c,d]Values are means ± SE. Values in the same row with different superscripts are different ($P < 0.05$).
[e]Feed intake was measured per pen. There were four piglets per pen and four pens per dietary treatment.

Table 4. Short-chain fatty acids (SCFA)[a] and lactic acid in the jejunum and cecum of piglets fed a control (CTR) diet with or without tributyrin (TRB) and(or) lactitol (LCT)

Item	Short-chain fatty acids, μmol/g DM						Total SCFA μmol/g DM	Lactic acid μmol/g DM
	Acetic acid	Propionic acid	Iso-butyric acid	n-butyric acid	Iso-valeric acid	*n*-valeric acid		
Jejunum[b]								
CTR	29.03 ± 4.9	3.98 ± 2.3	0.35 ± 0.3	2.34 ± 1.5	—[c] ± —	— ± —	35.70 ± 8.9	441.9 ± 45.4[de]
TRB	27.80 ± 15.1	7.77 ± 7.7	0.78 ± 0.8	7.83 ± 3.9	— ± —	— ± —	44.17 ± 25.7	201.7 ± 37.9[d]
LCT	26.68 ± 1.1	1.55 ± 1.5	1.47 ± 1.1	0.83 ± 0.8	— ± —	— ± —	30.53 ± 3.1	611.2 ± 124.4[e]
TRB+LCT	14.73 ± 2.8	— ± —	— ± —	5.47 ± 2.5	— ± —	— ± —	20.20 ± 4.9	243.7 ± 83.4[d]
Cecum[b]								
CTR	1,039 ± 193.8	701.4 ± 104.9	7.22 ± 3.2	332.3 ± 72.9	7.89 ± 5.2	98.55 ± 21.6	2,186 ± 377.5	70.72 ± 46.4[d]
TRB	640.0 ± 116.8	366.8 ± 76.4	3.76 ± 2.2	169.7 ± 70.2	2.69 ± 1.8	29.25 ± 15.3	1,212 ± 254.0	41.77 ± 24.7[d]
LCT	817.4 ± 156.1	500.4 ± 78.4	1.74 ± 1.7	268.0 ± 87.4	— ± —	40.99 ± 21.6	1,629 ± 323.7	213.2 ± 23.1[e]
TRB+LCT	671.1 ± 78.1	395.6 ± 58.1	3.15 ± 1.1	189.4 ± 37.5	— ± —	36.18 ± 14.2	1,295 ± 156.7	12.03 ± 12.1[d]

[a]SCFA = short-chain fatty acids, not including lactic acid.
[b]Values are means ± SE, n = 4.
[c]Below detection threshold.
[d,e]Values in the same column and in the same intestinal site with different superscripts are different ($P < 0.05$).

0.8 μmol/g DM, respectively; n = 32). Neither the tributyrin nor the lactitol diet reduced histamine concentrations in the jejunum or in the cecum. The only responses ($P < 0.05$) were 66 and 49% decreases in histamine levels in the jejunum and cecum, respectively, in animals fed the tributyrin+lactitol diet compared to the control diet (Table 5).

Scanning Electron Microscopy on Intestinal Mucosa

In the jejunum, both the lactitol and tributyrin + lactitol diets increased ($P < 0.001$) the mucosal thickness (Figure 1a) and villus length (Figure 1b) by about 18 and 12%, respectively, compared to the control and tributyrin diets. The tributyrin diet did not have any effect in the jejunum ($P > 0.05$) as determined by scanning electron microscopy. In addition, there were no differences ($P > 0.05$) in the length of the enterocytes among the dietary treatments (average length: 21.0 ± 4.2 μm with at least 25 measurements per dietary treatment).

In the cecum, the tributyrin, lactitol, and tributyrin + lactitol diets were effective in reducing the thickness of the mucosa (Figure 2a; $P < 0.001$) and the depth of the crypts (Figure 2b; $P < 0.001$) relative to the control diet. The lactitol diet reduced these parameters by 15 and 18%, respectively, whereas both the tributyrin and tributyrin+lactitol diets reduced ($P < 0.001$) the thickness of the mucosa and depth of the crypts by 26 and 45%, respectively, compared with the control diet.

Discussion

The ban on the use of certain antibiotic feed supplements instituted or planned in several European countries has resulted in a renewed interest in exploring alternative strategies to the use of antibiotic growth promoters. The search for alternatives to antibiotics has spawned the biotherapeutic, or "biotic," approach to enhancing animal health and performance. There have already been several reports published on the application of probiotic cultures, alone or in combination with prebiotic oligosaccharides, for improving the microbial balance in the gastrointestinal tract and, in so doing, favorably affecting the host (Howard et al., 1993; Monsan and Paul, 1995; Tannock, 1999). There have also been a few reports on the development of flavorants and herbal extracts for stimulating appetite, as well as for displaying antagonism toward undesirable microbes and improving the antioxidant status of the host and, in so doing, acting as growth promoters for swine (Luchansky, 2000; Piva, 2000). In following the biotic approach to animal husbandry and in the absence of antibiotic control of microbial degradation of nutrients for the host, a deeper understanding of the relationship between nutrient requirements for the animal and particularly for specific tissues, such as the gut, and the ecology of intestinal microbes is needed. Nutrients could be targeted for nourishing

Table 5. Mono-, di-, and polyamines (μmol/g DM) in the jejunum and cecum of piglets fed a control (CTR) diet with or without tributyrin (TRB) and/or lactitol (LCT)

Item	Tyramine	Cadaverine	Histamine	Putrescine	Spermidine	Spermine
Jejunum[a]						
CTR	2.04 ± 1.01	1.21 ± 0.60	2.81 ± 0.39[b]	0.68 ± 0.28	0.36 ± 0.08	1.06 ± 0.12
TRB	1.18 ± 0.62	1.76 ± 1.18	2.45 ± 0.31[bc]	0.68 ± 0.33	0.38 ± 0.10	1.58 ± 0.96
LCT	1.97 ± 0.51	1.63 ± 0.36	2.90 ± 0.35[b]	0.78 ± 0.08	0.43 ± 0.12	1.33 ± 0.28
TRB+LCT	1.83 ± 1.06	1.67 ± 0.41	0.95 ± 0.32[c]	0.98 ± 0.31	0.54 ± 0.12	0.88 ± 0.21
Cecum[a]						
CTR	1.34 ± 0.20	4.32 ± 1.46	2.97 ± 0.39[b]	4.25 ± 0.71	1.37 ± 0.23	1.02 ± 0.14
TRB	0.87 ± 0.24	4.39 ± 1.97	1.66 ± 0.17[bc]	4.46 ± 1.96	1.40 ± 0.33	0.93 ± 0.16
LCT	1.54 ± 0.31	5.31 ± 1.04	2.31 ± 0.19[bc]	4.55 ± 1.02	1.52 ± 0.14	0.97 ± 0.21
TRB+LCT	0.72 ± 0.25	2.98 ± 1.24	1.51 ± 0.41[c]	3.41 ± 1.12	2.14 ± 0.12	0.91 ± 0.26

[a]Values are means ± SE, n = 4.
[b,c]Values in the same column and in the same intestinal site with different superscripts are different ($P < 0.05$).

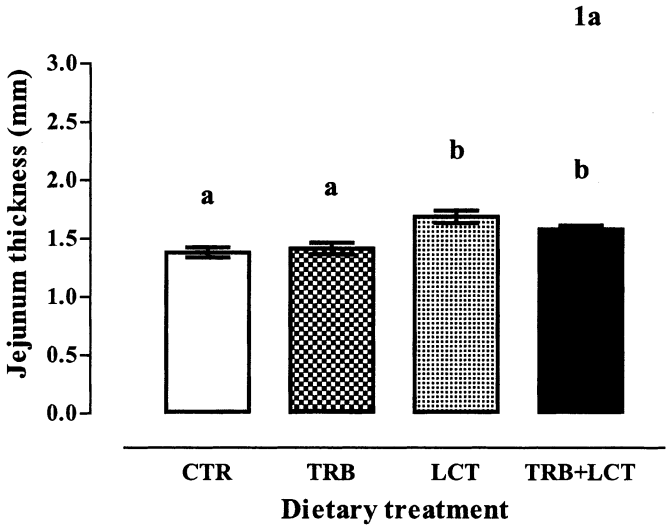
specific tissues. In particular, the gut responds positively to various compounds, defined as nutribiotics, such as SCFA, butyrate, glutamine, putrescine, and(or) α-ketoglutaric acid, which favorably affect the host by directly improving the overall trophism, as well as the digestion and absorption of nutrients (Luchansky, 2000; Pluske, 2001). The purpose of the present work was to evaluate tributyrin and lactitol as dietary and fermentable sources of butyrate, respectively, for improving swine health.

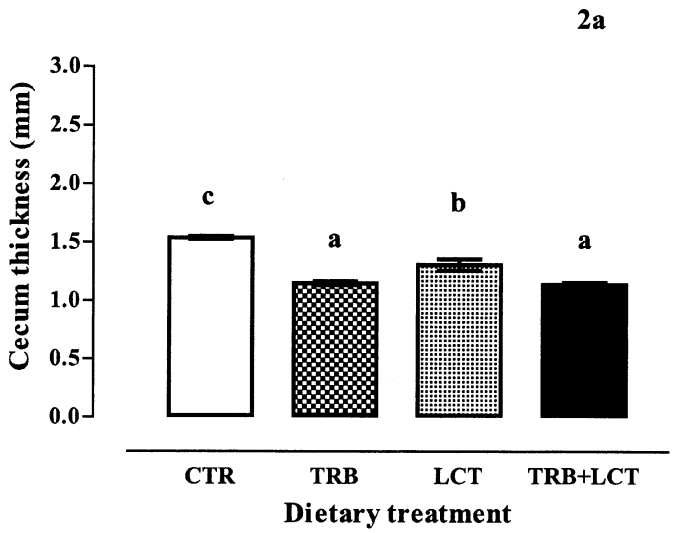
Animals receiving the control diet were affected by the stress conditions employed in this study. Records from the production barn for a 5-yr period using piglets of the same genotype revealed that piglets displayed an ADG of 180 and 400 g/d for d 0 to 14 and 0 to 42 on test, respectively, and a gain-to-feed ratio of 0.77 and 0.63 for d 0 to 14 and 0 to 42 on test, respectively. In the present study, only animals receiving both tributyrin and lactitol in the diet approached these performance levels. Moreover, a higher number of animals in the control group lost weight after d 14 of the feeding trial, and this resulted in a wider variance in the results for the various growth parameters than the experimental groups. Overall, although Bartlett's test for equal variances did not reach the significance level, the power of the statistical analyses was partially reduced due to the variable response of the control and tributyrin-fed animals. A larger number of animals would be necessary to provide sufficient statistical power to draw conclusions based on morbidity and(or) mortality, especially given the observed wide variances in the growth parameters displayed by animals fed the control diet.

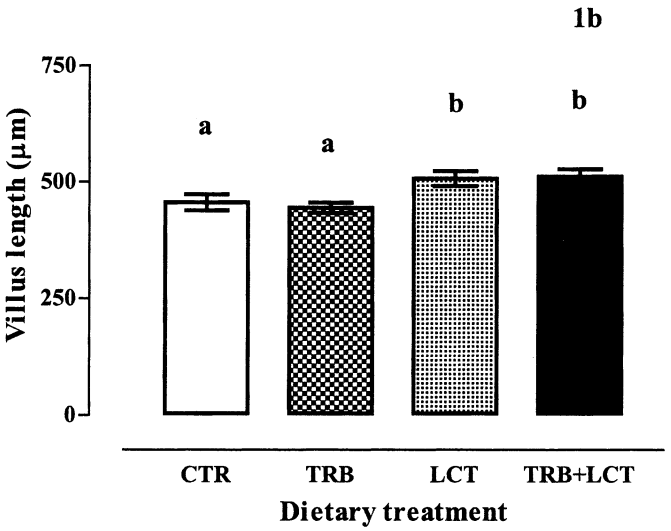
In this study, despite the lower mortality observed in animals fed the tributyrin diet compared to the control diet, tributyrin reduced the feed efficiency by 23% and reduced the total weight of weaned piglets by 7.5% after 42 d on test (163.6 vs 176.8 kg for the tributyrin and control groups, respectively). It is possible that some of the negative effects observed for these growth parameters could be attributed to the dose of tributyrin tested. Subsequent trials should evaluate doses of tributyrin that are less than 10 g/kg. Nevertheless, the tributyrin diet did not alter any other luminal parameter measured in the jejunum or in the cecum.

Animals fed the lactitol diet did not display improved growth performance. A growth-stimulating effect of lactitol was observed by other investigators when feeding lactitol alone (Harju et al., 1988) or in combination with lactulose (Nousiainen and Setälä, 1992). Body weight gains were decreased in a dose-related manner when lactitol (> 5%) was fed to rats (Sinkeldam et al., 1992). At the end of the present study, animals fed the lactitol diet displayed a 21.5% higher total live weight than animals fed the control diet (214.8 vs 176.8 kg for the lactitol and control groups, respectively) as a result of having more surviving animals. The lactitol diet did not modify the concentrations of SCFA but increased lactic acid levels in the jejunum and cecum. This is most likely an indication of an increased metabolism and proliferation of the indigenous population of lactic acid bacteria with its associated benefits for the host (Underdahl et al., 1982; Piva and Morelli, 1997; Kelly and King, 2001).

Compared to animals fed the control, tributyrin, or lactitol diets, animals fed the tributyrin+lactitol diet displayed the most desirable outcomes for all of the parameters measured. These animals experienced no weight loss and no mortality during the 42-d feeding period. These animals also showed an improved ADG and feed efficiency, and achieved a 34% higher total live weight than animals fed the control diet at the end of the study (237.4 vs 176.8 kg for the tributyrin+lactitol and control groups, respectively). The higher lactic acid concentrations found in the jejunum and cecum of animals fed the lactitol diet were reduced to the levels observed with the control diet when lactitol was fed in combination with tributyrin. It is notable that the tributyrin+lactitol diet was particularly effective at controlling histamine levels both in the jejunum and in the cecum. The release of hista-







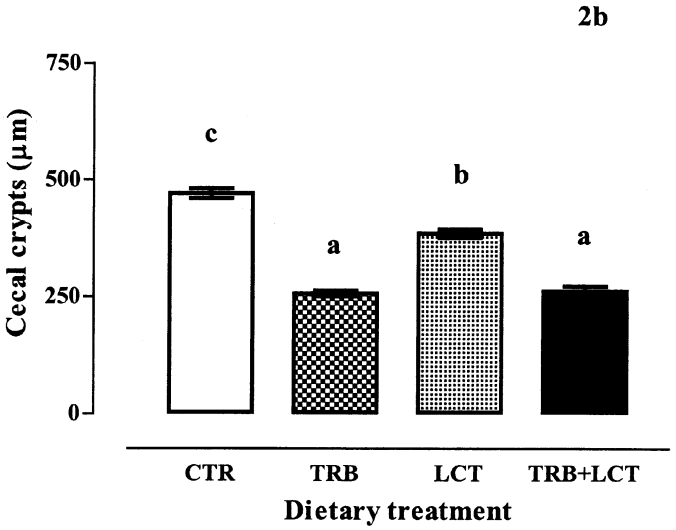


Figure 1. Jejunum thickness in piglets fed a control (CTR) diet with or without tributyrin (TRB, 10 g/kg) and(or) lactitol (LCT, 3 g/kg). a) Thickness was measured by scanning electron microscopy recording at least 20 measurements per dietary treatment. b) Villus length in jejunum of piglets, measured by scanning electron microscopy recording at least 24 measurements per treatment. Data were compared with one-way ANOVA using the Newman-Keuls posttest. Error bars indicate SE. Columns with different superscript letters differ ($P < 0.05$).

Figure 2. Cecum thickness in piglets fed a control (CTR) diet with or without tributyrin (TRB, 10 g/kg) and(or) lactitol (LCT, 3 g/kg). a) Thickness was measured by scanning electron microscopy recording at least 28 measurements per treatment. b) Crypt depth, measured by scanning electron microscopy recording at least 24 measurements per treatment. Data were compared with one-way ANOVA using the Newman-Keuls posttest. Error bars indicate SE. Columns with different superscript letters differ ($P < 0.001$).

mine by mast cells exhibits various biological effects related to allergic enteropathy, inflammatory bowel disease (Raithel et al., 1995), and stress-related gut dysfunction (Santos et al., 1998). Histamine lowers the blood pressure by dilating blood vessels and causes inflammatory reactions by promoting leukocyte chemotaxis (Mitsuoka, 1993). Histamine is also associated with increased colonic secretion (Wang et al., 1990) and ileum contraction (Bartho et al., 1987), as well as with celiac disease by displaying atrophy of villi, hyperplasia of crypts, and increase of mucosal volume (Wingren et al., 1986). As such, feeding the tributyrin+lactitol diet may be beneficial for limiting the exposure of the gut to proinflammatory conditions. The concentrations of the other amines analyzed were within physiological ranges (Bardocz et al., 2001) with no differences among the various dietary treatments. Putrescine, spermidine, and spermine act as mediators of the histological development of the gut cells

(Bardocz et al., 1998) and may originate from the body pool, diet, and intestinal fermentation. Cadaverine, putrescine, spermidine, histamine, and tyramine can also result from intestinal bacterial decarboxilation (Mitsuoka, 1993). In this study, the increased concentration of cadaverine, putrescine, and spermidine in the cecum compared to the jejunum confirms the higher physiological rate of bacterial proteolysis occurring in the large intestine that may be attributed to the lack of fermentable energy reaching the hindgut (Russell et al., 1983).

Both the lactitol and tributyrin+lactitol diets resulted in an increase in the length of the villi (+13%) compared with control and tributyrin diets. Such a modification of the mucosal structure has been positively correlated with the rate of body weight gain (Pluske et al., 1996a), feed intake (Pluske et al., 1996b), and nutrient digestibility (Pluske et al., 1996c). It was also observed when lactose and milk products were fed to piglets during the postweaning phase (Mahan, 1992).

The use of tributyrin or lactitol as a dietary or fermentable source, respectively, of *n*-butyric acid did not result in a measurable increase of butyrate in the jejunum or cecum. This could be explained, at least in part, because this study was not designed to measure concentrations of butyrate in portal blood or gut tissues and, as such, we were unable to quantify the total amount of butyrate resulting from the diets tested. However, in a previous study (Piva et al., 1996), we found that when added to low- and high-fiber diets, lactitol increased the concentration of *n*-butyrate to 142 and 230%, respectively, in an in vitro swine cecal fermentation. By design, this in vitro system precluded the absorption of SCFA and allowed us to measure accumulated *n*-butyrate (Piva et al., 1996). In the present study, the butyric acid originating from fermented lactitol or from tributyrin could not be measured at slaughter due to the rapid absorption rate of *n*-butyrate by the intestinal mucosa. However, the shorter crypt depths observed for animals fed the lactitol diet (–18% vs the control diet) and for the tributyrin and tributyrin+lactitol diets (–45% vs the control diet) support the hypothesis that additional *n*-butyric acid was available and that such levels had an antiproliferative effect in piglets, as proposed by von Engelhardt et al. (1998). In fact, *n*-butyric acid was previously shown to reduce by 60% the number of proliferating cells in the upper 40% of crypts (Scheppach et al., 1997). It also resulted in a concomitant reduction of blood discharge and inflammation (Scheppach et al., 1992). In the present study, tributyrin had a more potent effect than *n*-butyric acid produced in the cecum from lactitol in reducing crypt growth. Accordingly, tributyrin was more potent than *n*-butyric acid in suppressing cell growth and promoting differentiation of colon cancer cells (Chen and Breitman, 1994; Schroder et al., 1998).

The shorter cecal crypts observed in the present study indicate that less tissue proliferation occurred in the hindgut and that less energy was allocated to the gut tissue, suggesting an energy-sparring effect for growth. In fact, the gastrointestinal tract has an average consumption of approximately 15 to 25% of all incoming energy, with a daily rate of visceral protein synthesis ranging from 8 to 185% (Cant et al., 1996). Under such conditions, the benefit of the increased absorption capacity of the intestine outweighs the added energy expenditure to achieve such capacity (Cant et al., 1996). However, in the growing animal, there reaches a point at which the allocation of a greater fraction of metabolizable energy to the intestine for improved absorption capacity is detrimental to the overall efficiency of energy utilization for growth (Cant et al., 1996). The mucosal structure with longer villi and shorter cecal crypts observed in animals fed the lactitol or the tributyrin+lactitol diets supports the hypothesis of better nutrient absorption in the small intestine with the least energy-demanding configuration for the hindgut. Similar results, such as reduced mucosal thickness, had been observed in antibiotic-fed or germ-free animals (Nousiainen, 1991; Visek, 1978).

Considerable research is being conducted to develop, optimize, and implement nonpharmaceutical approaches for enhancing animal wellness and performance. To this end, we provide the first report of the use of tributyrin, in combination with lactitol, as a nutribiotic feed supplement to facilitate the transition to solid feed in swine. These data validate the concept of directly feeding gut tissue to achieve a desirable growth response. More specifically, our results indicate that tributyrin and lactitol act synergistically to prevent the stress-associated postweaning syndrome in piglets, to establish a more digestion-efficient structure of the mucosa, and to reduce proinflammatory conditions of the gastrointestinal tract. Further studies should focus on the identification of the most effective and safe dose, and ratio of tributyrin to lactitol in nursery pigs.

Implications

Husbandry practices, diet, and(or) various environmental stresses can greatly influence the well-being of animals. Antibiotics have been used to ameliorate such negative influences. Since the World Health Organization proposal in the last decade for a global reduction or elimination of antibiotics in animal feed, there has been renewed interest in exploring alternative growth enhancers. The results of the present study validate the efficacy of a combination of tributyrin and lactitol to facilitate the transition from milk to solid feed in the gastrointestinal tract of nursery pigs. These data also demonstrate that feeding this combination is more beneficial to swine than feeding either tributyrin or lactitol alone for enhancing the trophic status of the intestinal mucosa and reducing histamine levels in the gut.

Literature Cited

AOAC. 1990. Official Methods of Analysis. 15th ed. Association of Official Analytical Chemists, Arlington, VA.

Bardocz, S., G. Grant, D. S. Brown, and A. Pusztai. 1998. Putrescine as a source of instant energy in the small intestine of the rat. Gut 42:24–28.

Bardocz, S., A. White, G. Grant, T. J. Walker, D. S. Brown, and A. Pusztai. 2001. The role of polyamines in intestinal function and gut maturation. In: A. Piva, K. E. Bach Knudsen, and J. E. Lindberg. (ed.) Gut Environment of Pigs. pp 29–42. Nottingham University Press, Nottingham, UK.

Bartho, L., G. Petho, A. Antal, P. Holzer, and J. Szolcsanyi. 1987. Two types of relaxation due to capsaicin in the guinea pig isolated ileum. Neurosci. Lett. 81:146–150.

Cant, J. P., B. W. McBride, and W. J. Croom, Jr. 1996. The regulation of intestinal metabolism and its impact on whole animal energetics. J. Anim. Sci. 74:2541–2553.

Chen, Z. X., and T. R. Breitman. 1994. Tributyrin: a prodrug of butyric acid for potential clinical application in differentiation therapy. Cancer Res. 54:3494–3499.

CFR. 1997. Code of Federal Regulations. Food and Drugs, 21, Parts 170 to 199, 184.1903. Office of the Federal Register National Archives and Records Administration, Washington, DC.

CFR. 2000. Code of Federal Regulations. Food and Drugs, 21, Parts 170 to 199, 101.80. Office of the Federal Register National Archives and Records Administration, Washington, DC.

Flores, H. E., and A. Galston. 1982. Analysis of poly-amines in higher plants by high performance liquid chromatography. Plant Physiol. (Rockv.) 69:701–706.

Fussel, R. J., and D. V. McCailey. 1987. Determination of volatile fatty acids (C2–C5) and lactic acid in silage by gas-chomatography. Analyst 112:1213–1216.

Harju, M. E., J. J. Setälä, M. K. Heikonen, and E. P. Linko. 1988. Growth-stimulating animal feed, a process for preparing it, and an additive to be used in it. United States Patent No. 4,760,055. Available at: http://www.uspto.gov/web/offices/pac/dapp/pac/pac.html. Accessed Oct. 2, 2001.

Howard, M. D., D. T. Gordon, L. W. Pace, K. A. Garleb, and M. S. Kerley. 1993. Effects of dietary supplementation with fructooligosaccharides on colonic microbiota populations and epithelial cell proliferation in neonatal pigs. J. Pediatr. Gastroenterol. Nutr. 21:297–303.

Kelly, D., and T. P. King. 2001. Luminal bacteria: Regulation of gut function and immunity. In: A. Piva, K. E. Bach Knudsen, and J. E. Lindberg. (ed.) Gut Environment of pigs. pp 113–131. Nottingham University Press, Nottingham, UK.

Levrat, M. A., C. Rémésy, and C. Demigné. 1991. High propionic acid fermentations and mineral accumulation in the cecum of rats adapted to different levels of inulin. J. Nutr. 121:1730–1737.

Luchansky, J. B. 2000. Use of biotherapeutics to enhance animal well being and food safety. In: G. Piva and F. Masoero. (ed.) Proc. 6th Int. Feed Prod. Conf., Piacenza, Italy. pp 188–194.

Luchansky, J. B., and A. Piva. 2001. Feed additive that increases availability of butyric acid and non-digestible oligosaccharides in the G.I. tract. United States Patent No. 6,217,915. Available at: http://www.uspto.gov/web/offices/pac/dapp/pac/pac.html. Accessed Oct. 2, 2001.

Mahan, D. C. 1992. Efficacy of dried whey and its lactalbumin and lactose components at two dietary lysine levels on post-weaning pig performance and nitrogen balance. J. Anim. Sci. 70:2182–2187.

Mitsuoka, T. 1993. Intestinal bacteria and health. 146–148. Harcourt Brace Jovanovich Japan, Inc., Ichibancho Central Bldg., 22-1, Chiyoda-ku, Tokyo.

Monsan, P. F., and F. Paul. 1995. Oligosaccharide feed additives. In: P. J. Wallace and A. Chesson (ed.) Biotechnology in Animal Feeds and Animal Feeding. pp 233–245. VCH Publishers, New York.

Morgan, C. A., and C. T. Whittemore. 1982. Energy evaluation of feeds and compounded diets for pigs. A review. Anim. Feed Sci. Technol. 7:387–400.

Noblet, J., H. Fortune, X. S. Shi, and S. Dubois. 1994. Prediction of net energy value of feeds for growing pigs. J. Anim. Sci. 72:344–354.

Nousiainen, J. T. 1991. Comparative observations on selected probiotics and olaquindox as feed additives for piglets around weaning. 2. Effect on villus length and crypt depth in the jejunum, ileum, caecum and colon. J. Anim. Physiol. Anim. Nutr. 66:224–230.

Nousiainen, J. T., and J. K. Setälä. 1992. Feed for promoting the growth and intestinal function of animals. European Patent 0 464 362 A1. Available at: http://www.european-patent-office.org. Accessed Oct. 2, 2001.

Partanen, K. H., and Z. Mroz. 1999. Organic acids for performance enhancement in pig diets. Nutr. Res. Rev. 12:117–145.

Piva, A. 2000. Alternatives to antibiotics. In: Proc. Eastern Nutr. Conf., Montreal, Canada. pp 81–101.

Piva, A., and L. Morelli. 1997. Role of swine isolated *Lactobacillus reuteri* and *L. acidophilus* on the microbial ecology and fermentation pattern in the gastro-intestinal tract of the adult pig. Zootecnia Nutr. Anim. 23:147–155.

Piva, A., A. Panciroli, E. Meola, and A. Formigoni. 1996. Lactitol enhances short-chain fatty acid and gas production by swine cecal microflora to a greater extent when fermenting low rather than high fiber diets. J. Nutr. 126:280–289.

Pluske, J. R. 2001. Morphological and functional changes in the small intestine of the newly-weaned pig. In: A. Piva, K. E. Bach Knudsen, and J. E. Lindberg. (ed.) Gut Environment of Pigs. pp 1–27. Nottingham University Press, Nottingham, UK.

Pluske, J. R., M. J. Thompson, C. S. Atwood, P. H. Bird, I. H. Williams, and P. E. Hartmann. 1996a. Maintenance of villous height and crypt depth, and enhancement of disaccharide digestion and monosaccharide absorption, in piglets fed on cows' whole milk after weaning. Br. J. Nutr. 76:409–422.

Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996b. Maintenance of villous height and crypt depth in piglets by providing continuous nutrition after weaning. Anim. Sci. 62:131–144.

Pluske, J. R., I. H. Williams, and F. X. Aherne. 1996c. Villous height and crypt depth in piglets in response to increases in the intake of cows' milk after weaning. Anim. Sci. 62:145–158.

Raithel, M., M. Matek, H. W. Beankler, W. Jorde, and E. G. Hahn. 1995. Mucosal histamine content and histamine secretion in Crohn's disease, ulcerative colitis and allergic enteropathy. Int. Arch. Allergy Immunol. 108:127–133.

Roediger, W. E. 1980. Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut 21:793–798.

Russell, J. B., C. J. Sniffen, P. J. Van Soest. 1983. Effect of carbohydrate limitation on degradation and utilization of casein by mixed rumen bacteria. J. Dairy Sci. 66:763–775.

Santos, J., E. Saperas, C. Nogueiras, M. Mourelle, M. Antolin, A. Cadahia, and J. R. Malagelada. 1998. Release of mast cell mediators into the jejunum by cold pain stress in humans. Gastroenterology 114:640–648.

Scheppach, W., J. G. Muller, F. Boxberger, G. Dusel, F. Richter, H. P. Bartram, S. U. Christl, C. E. Dempfle, and H. Kasper. 1997. Histological changes in the colonic mucosa following irrigation with short-chain fatty acids. Eur. J. Gastroenterol. Hepatol. 9:163–168.

Scheppach, W., H. Sommer, T. Kirchner, G. M. Paganelli, P. Bartram, S. Christl, F. Richter, G. Dusel, and H. Kasper. 1992. Effect of butyrate enemas on the colonic mucosa in distal ulcerative colitis. Gastroenterology 103:51–56.

Schroder, C., K. Eckert, and H. R. Maurer. 1998. Tributyrin induces growth inhibitory and differentiating effects on HT-29 colon cancer cells in vitro. Int. J. Oncol. 13:1335–1340.

Schroder, O., S. Hess, W. F. Caspary, and J. Stein. 1999. Mediation of differentiating effects of butyrate on the intestinal cell line Caco-2 by transforming growth factor-beta 1. Eur. J. Nutr. 38:45–50.

Seiler, N., and B. Knodgen. 1978. Determination of di- and polyamines by high performance liquid chromatographic separation of their dimethylaminonaphthalene-1-sulfonyl-derivatives. J. Chromatogr. 145:29–39.

Sinkeldam, E. J., R. A. Woutersen, V. M. H. Hollanders, H. P. Til, A. Van Garderen-Hoetmer, and A. Bär. 1992. Subchronic and chronic toxicity/carcinogenicity feeding studies with lactitol in rats. J. Am. Coll. Toxicol. 11:165–188.

Tannock, G. W. 1999. Probiotics: a critical review. Horizon Scientific Press, Wymondham, UK.

Tsukamura, M., H. Goto, T. Arisawa, T. Hayakawa, N. Nakai, T. Murakami, N. Fujitsuka, and Y. Shimomura. 1998. Dietary mannitol decreases the incidence of 1,2-dimethylhydrazine-induced cecum and proximal colon tumors in rats. J. Nutr. 128:536–540.

Underdahl N., A. Torres, and A. Doster. 1982. Effect of *Streptococcus faecium* C-68 in control of *Escherichia coli* induced diarrhoea in gnotobiotic pigs. Am. J. Vet. Res. 43:2227–2232.

Visek, W. J. 1978. The mode of growth promotion by antibiotics. J. Anim. Sci. 46:1447–1469.

von Engelhardt, W., J. Bartels, S. Kirschberger, H. D. Meyer zu Düttingdorf, H. D., and R. Busche. 1998. Role of short-chain fatty acids in the hindgut. Vet. Q. 20:S52–S59.

Wang, Y. Z., H. J. Cooke, H. C. Su, and R. Fertel. 1990. Histamine augments colonic secretion in guinea pig distal colon. Am. J. Physiol. 258:432–439.

Wingren, U., C. Hallert, K. Norrby, and L. Enerback. 1986. Histamine and mucosal mast cells in gluten enteropathy. Agents Actions 18:266–268.